

Fig. 1

NR-LU-13 Heavy chain variable region sequences

GAG GTT CAG CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA GTC AGG TTG TCC TGC Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys	22
<u>CDR1</u>	
ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG GTG ATA GAG AGG CCT GAA CAG GGC Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Ile Glu Arg Pro Glu Gln Gly	44
<u>CDR2</u>	
CTG GAG TGG ATT GGA AGG ATT GAT CCT GCG AAT GGT AAT ACT AAA TGT GAC CCG AAG TTC CAG GGC Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Cys Asp Pro Lys Phe Gln Gly	66
<u>CDR3</u>	
AAG GCC ACT ATA ACA GCA GAC ACA TCC TCC AAC ACA GCA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser	88
<u>CDR3</u>	
GAG GAC ACT GCC GTC TAT TAC TGT TCT AGA GAG GTC CTA ACT GGG ACG TGG TCT TTG GAC TAC TGG Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp Tyr Trp	110
GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA Gly Gln Gly Thr Ser Val Thr Val Ser Ser	120

NR-LU-13 Light chain variable region sequences

GAC ATC CAG ATG ATT CAG TCT CCA TCG TCC ATG TTT GCC TCT CTG GGA GAC AGA GTC AGT CTC TCT Asp Ile Gln Met Ile Gln Ser Pro Ser Ser Met Phe Ala Ser Leu Gly Asp Arg Val Ser Leu Ser	22
<u>CDR1</u>	
TGT CGG GCT AGT CAG GGC ATT AGA GGT AAT TTA GAC TGG TAT CAG CAG AAA CCA GGT GGA ACT ATT Cys Arg Ala Ser Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr Gln Gln Lys Pro Gly Gly Thr Ile	44
<u>CDR2</u>	
AAA CTC CTG ATC TAC TCC ACA TCC AAT TTA AAT TCT GGT GTC CCA TCA AGG TTC AGT GGC AGT GGG Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Asn Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly	66
TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTA GAC TCT GAA GAT TTT GCA GAC TAT TAC TGT Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Ser Glu Asp Phe Ala Asp Tyr Tyr Cys	88
<u>CDR3</u>	
CTA CAG CGT AAT GCG TAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA Leu Gln Arg Asn Ala Tyr Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys	107

Light Chain

1	5	10							
ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER
11	15	20							
LEU	SER	ALA	SER	VAL	GLY	ASP	ARG	VAL	THR
21	25	30							
ILE	THR	CYS	ARG	ALA	SER	GLN	GLY	ILE	ARG
31	35	40							
GLY	ASN	LEU	ASP	TRP	TYR	GLN	GLN	LYS	PRO
41	45	50							
GLY	LYS	GLY	PRO	LYS	LEU	LEU	ILE	TYR	SER
51	55	60							
THR	SER	ASN	LEU	ASN	SER	GLY	VAL	PRO	SER
61	65	70							
ARG	PHE	SER	GLY	SER	GLY	SER	GLY	SER	ASP
71	75	80							
TYR	THR	LEU	THR	ILE	SER	SER	LEU	GLN	PRO
81	85	90							
GLU	ASP	PHE	ALA	THR	TYR	TYR	CYS	LEU	GLN
91	95	100							
ARG	ASN	ALA	TYR	PRO	TYR	THR	PHE	GLY	GLN
101	105								
GLY	THR	LYS	LEU	GLU	ILE	LYS			

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

Heavy Chain

1	5	10							
GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU
11	15	20							
VAL	LYS	LYS	PRO	GLY	ALA	SER	VAL	LYS	VAL
21	25	30							
SER	CYS	LYS	ALA	SER	GLY	PHE	ASN	ILE	LYS
31	35	40							
ASP	THR	TYR	MET	HIS	TRP	VAL	ARG	GLN	ALA
41	45	50							
PRO	GLY	GLN	GLY	LEU	GLN	TRP	MET	GLY	ARG
51	55	60							
ILE	ASP	PRO	ALA	ASN	GLY	ASN	THR	LYS	CYS
61	65	70							
ASP	LEU	SER	PHE	GLN	GLY	ARG	VAL	THR	ILE
71	75	80							
THR	ALA	ASP	THR	SER	ILE	ASN	THR	ALA	TYR
81	85	90							
MET	GLU	LEU	SER	SER	LEU	ARG	SER	ASP	ASP
91	95	100							
THR	ALA	VAL	TYR	TYR	CYS	SER	ARG	GLU	VAL
101	105	110							
LEU	THR	GLY	THR	TRP	SER	LEU	ASP	TYR	TRP
111	115	120							
GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

Alignment of the Light Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

DIQMISSPSSMFASLGDRVSLSC RASQGIRGNLD WYQQKPGGTIKLLIY STSNLNS

.....
DIQMTQPSSLSASVGDRVTITC RASQGIRGNLD WYQQKPGKGPKLLIY STSNLNS
CDR1 CDR2

GVPSRFSGSGSGSDYSLTISSLESEDFA~~D~~YYC LQRNAYPYTF GGGTKLEIK

.....
GVPSRFSGSGSGSDYTLTISSLQPEDFA~~D~~YYC LQRNAYPYTF GQG~~T~~KLEIK
CDR3

Alignment of the Heavy Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

EVQLQSGAELVKPCASVRLSCTASGFNIK DTYMH WVIERPEQGLEWIG

.....
QVQLVQSGAEVKPGASVKVSCKASGFNIK DTYMH WVRQAPGQGLQWMG
CDR1

RIDPANGNTK CDPKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCS

.....
RIDPANGNTK CDLSFQGRVTITADTSINTAYMELSSLRSDDTAVYYCS
CDR2

REVLTGTVSLDY WGQGTSVTVSS

.....
REVLTGTVSLDY WGQGTLVTVSS
CDR3

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY NR-LU-13 AND THEIR USE IN PRETARGETING METHODS
Inventor(s): Scott S. Graves et al. Express Mail No. EV336652109US Docket No. 690022.527C4

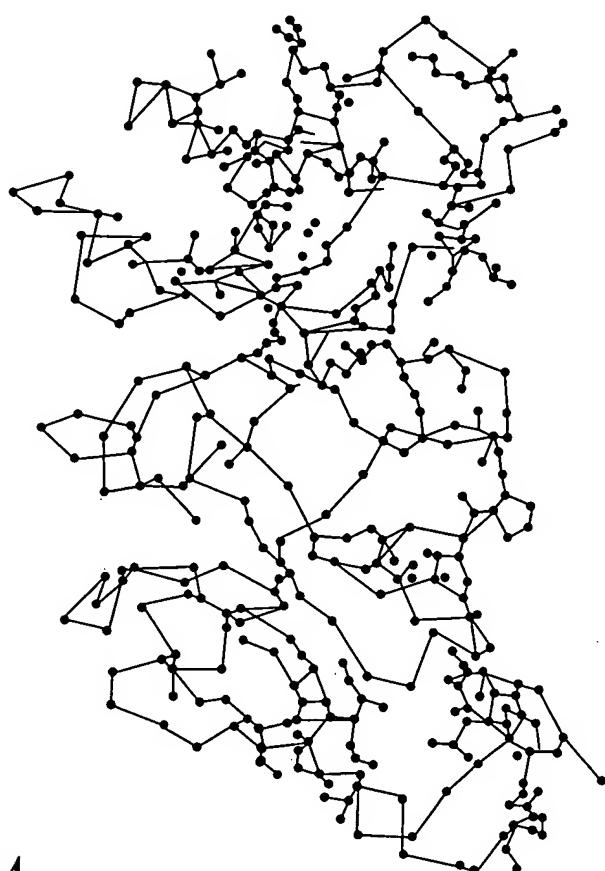


Fig. 6A

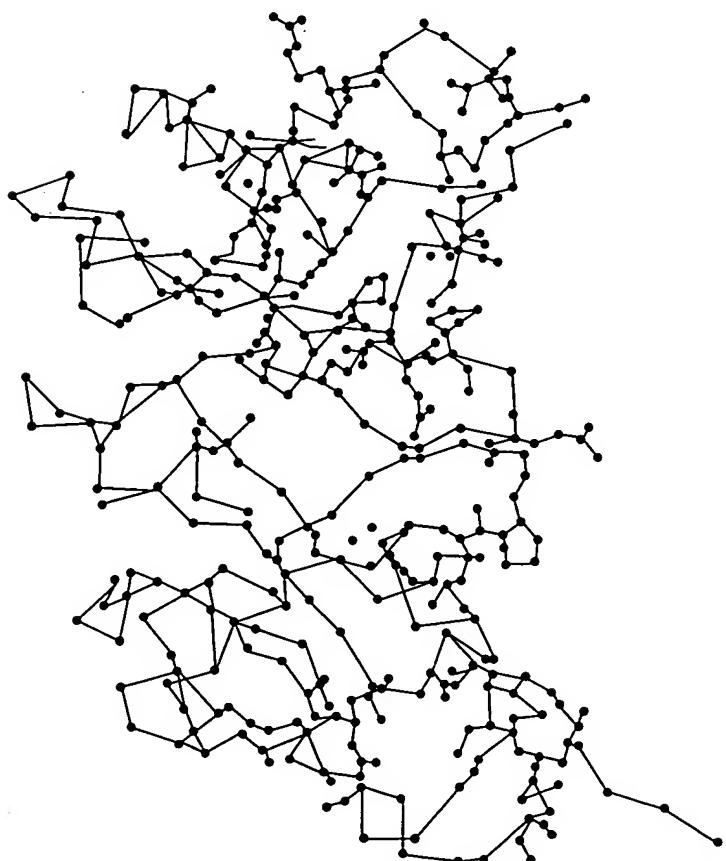


Fig. 6B

Same frequencies, but matching with human sequences. Only one occurrence of Asp at position 182 is found and no occurrences of Cys at position 181.

RES	181	182
A	-	0.48
R	-	0.02
N	0.01	0.18
D	0.00	0.00
C	0.00	0.00
Q	0.00	-
E	-	-
G	0.00	0.01
H	0.00	-
I	-	0.00
L	-	0.00
K	0.00	0.00
M	-	-
F	0.03	-
P	0.00	0.01
S	0.01	0.23
T	-	0.02
W	0.00	-
Y	0.91	-
V	0.00	0.02
X	0.01	0.02
O	-	-
-	-	-
Z	-	-
B	-	0.00
Total	1.00	1.00

Fig. 7A

Sequence positions 50 and 183 are structural mutations within 5 Å of the CDR's. Frequency of residue types at these positions are identical.

Position 50 (153 human lambda sequences)

RES	50
A	-
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	-
K	-
M	0.00
F	-
P	0.93
S	-
T	-
W	-
Y	-
V	-
X	0.06
O	-
-	-
Z	-
B	-
Total	1.00

Fig. 7B

Position 50 (279 human kappa sequences)

RES 50

A	0.00
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	0.00
K	-
M	-
F	-
P	0.96
S	-
T	-
W	-
Y	-
V	-
X	0.03
O	-
-	-
Z	-
B	-

Total 1.00

Fig. 7C

Position 50 is highly conserved in all the sequences, but proline can be exchanged by Ile or Leu. The framework used for the light chain (6fab) does have an Ile at this position. If this position is compared to other structures the backbone torsions are the same for structures with a Pro and an Ile at this position.

Position 50 (153 human lambda sequences)

RES	183
A	0.06
R	-
N	0.00
D	0.21
C	-
Q	0.15
E	0.01
G	0.01
H	-
I	0.00
L	0.00
K	0.00
M	-
F	0.00
P	0.40
S	0.01
T	0.01
W	-
Y	0.00
V	0.08
X	0.02
O	-
-	-
Z	-
B	0.00
Total	1.00

Fig. 7D

Position 183 (1210 mouse sequences)

RES 183

A	0.16
R	0.00
N	0.00
D	0.13
C	-
Q	0.16
E	0.25
G	0.02
H	0.00
I	-
L	-
K	0.00
M	-
F	-
P	0.17
S	0.08
T	0.00
W	-
Y	-
V	0.00
X	0.02
O	-
-	-
Z	-
B	-

Total 1.00

Leu is seen in human sequences at this position, but never in murine sequences, for both human and murine Sequences P is the most frequently occurring residue at position 183.

Fig. 7E

Comments for pcDNA3:
5446 nucleotides

CMV promotor: bases 209–863
T7 promotor: bases 864–882
Polylinker: bases 889–994
Sp6 promotor: bases 999–1016
BGH poly A: bases 1018–1249
SV40 promotor: bases 1790–2115
SV40 origin of replication: bases 1984–2069
Neomycin ORF: bases 2151–2945
SV40 poly A: bases 3000–3372
ColE1 origin: bases 3632–4305
Ampicillin ORF: bases 4450–5310

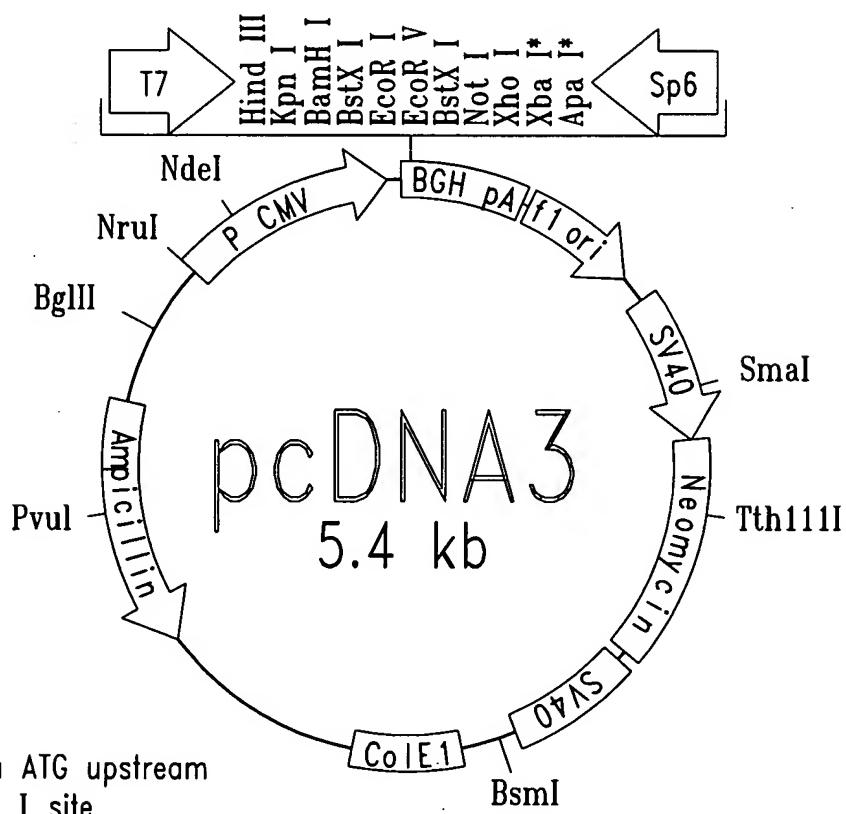


Fig. 8

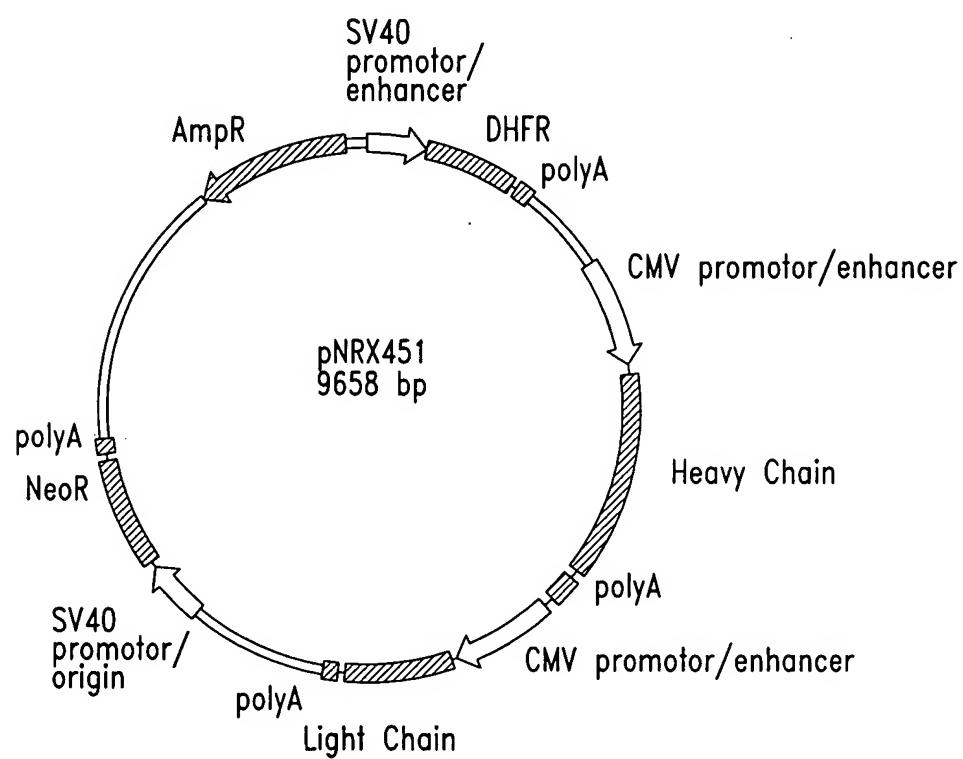


Fig. 9

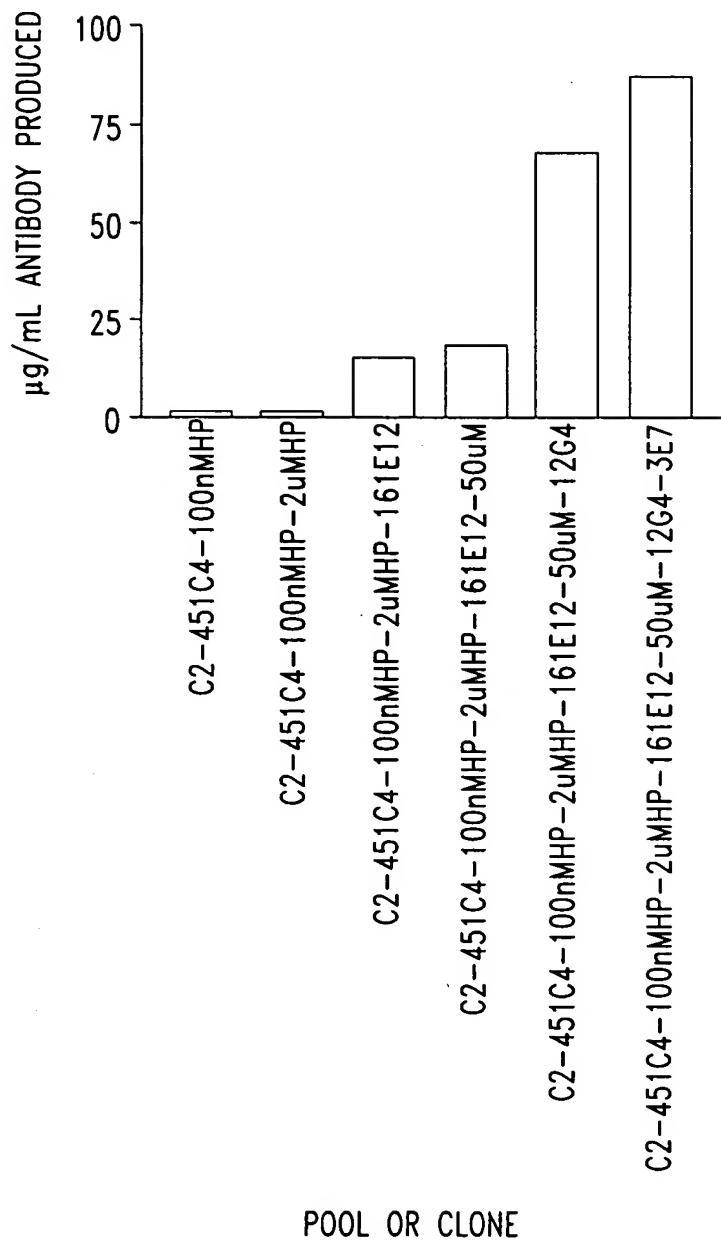


Fig. 10

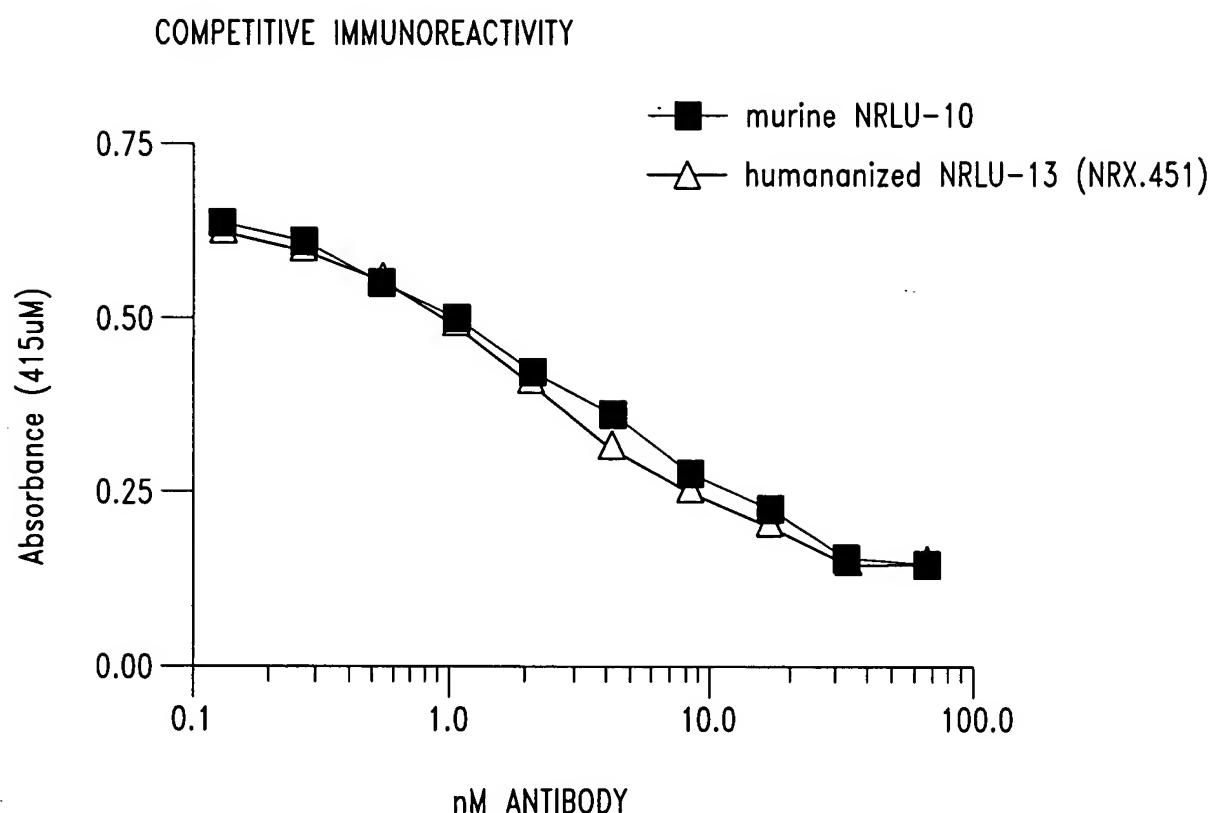


Fig. 11

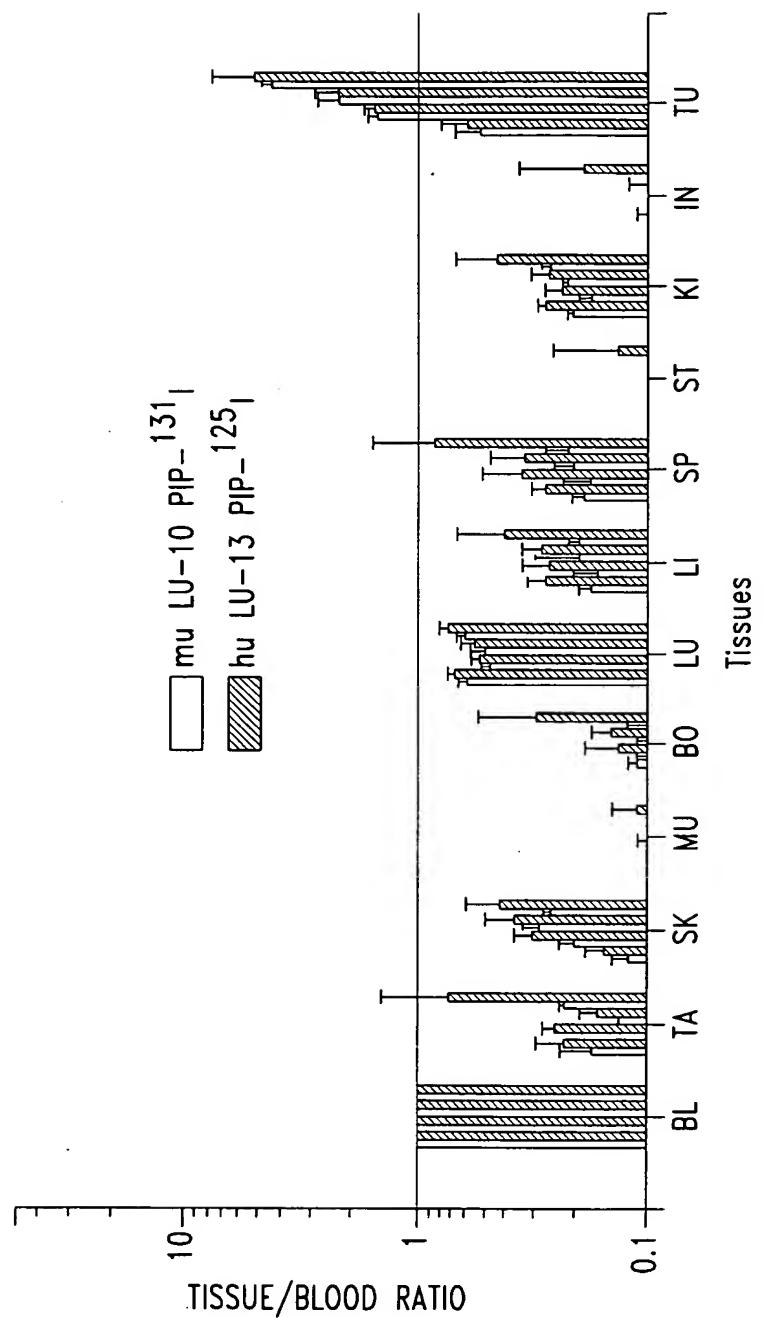


Fig. 12

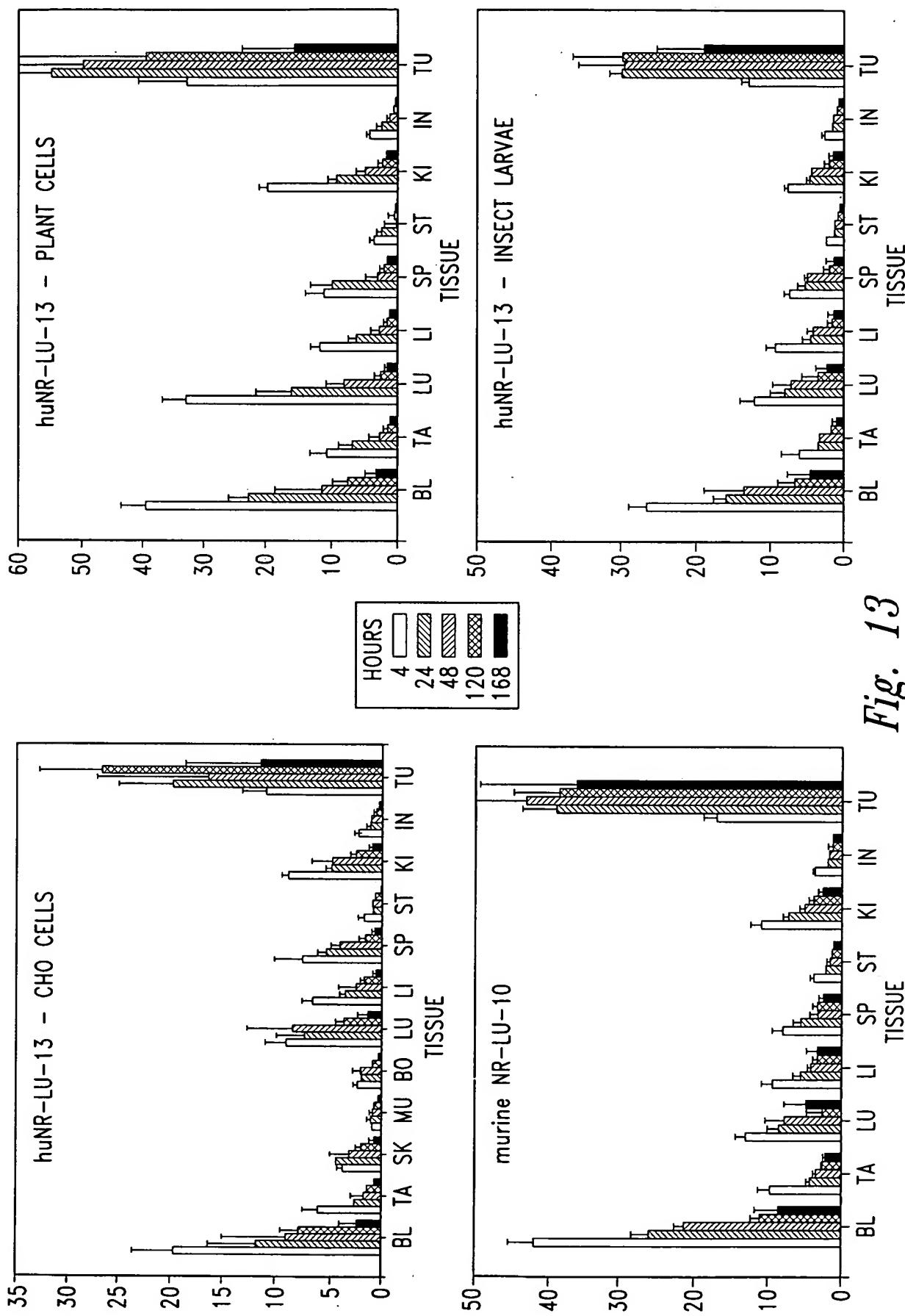


Fig. 13

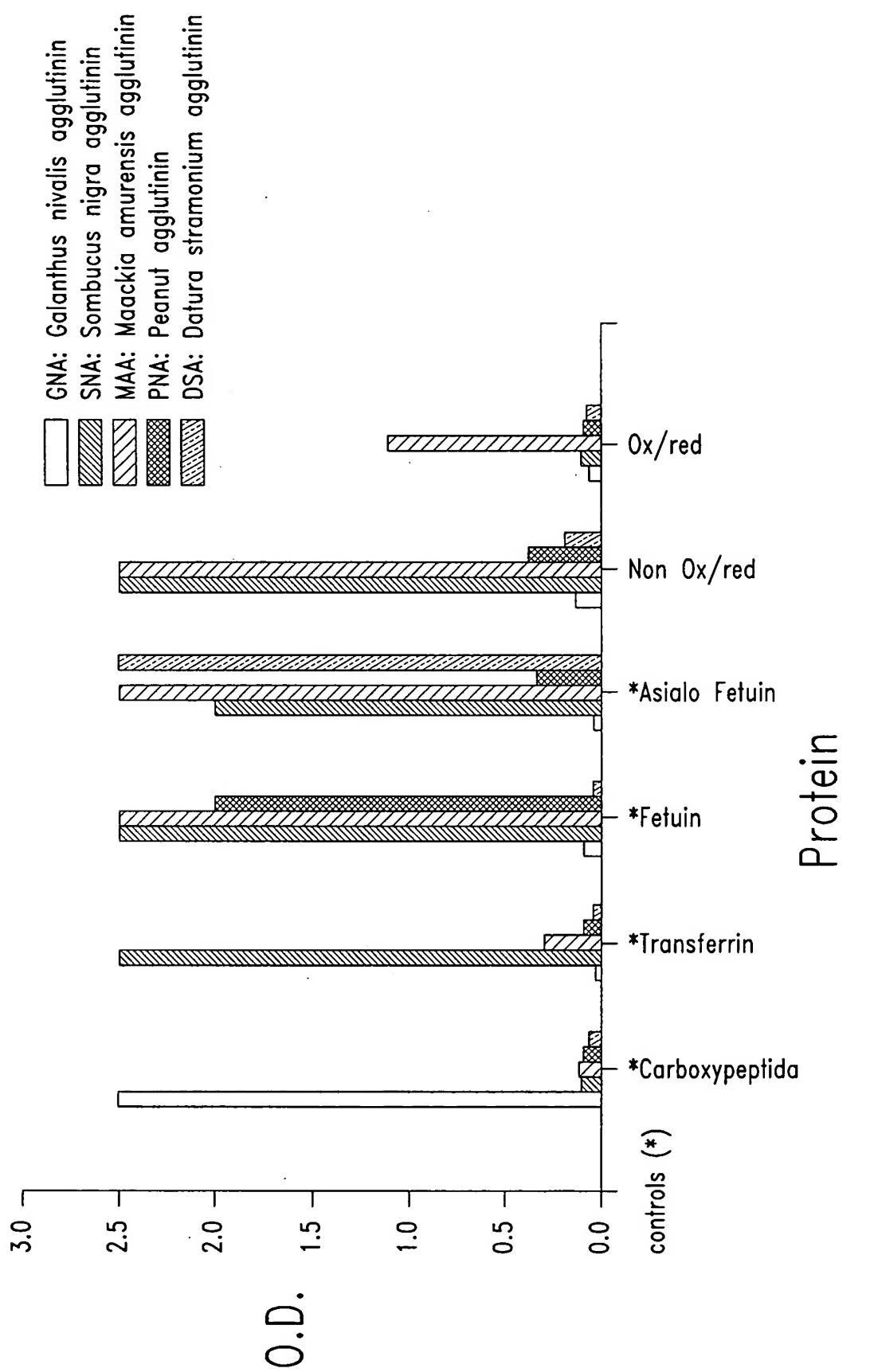


Fig. 14

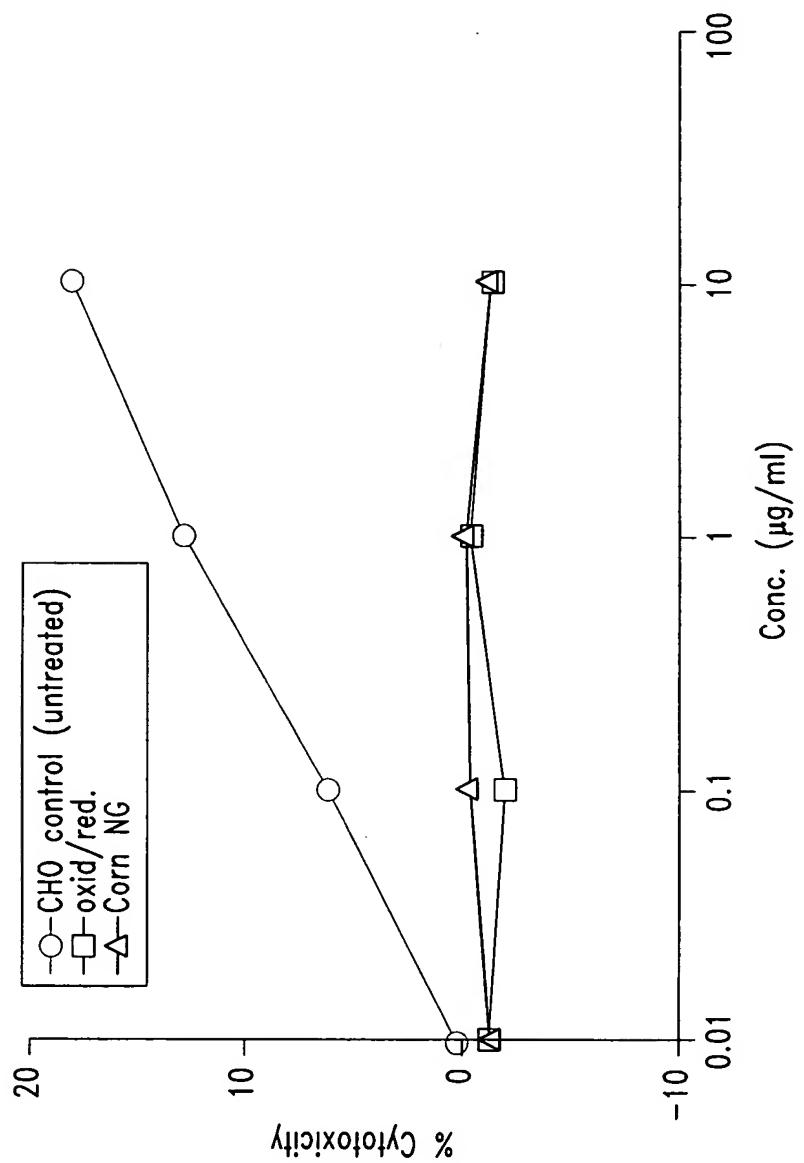


Fig. 15A

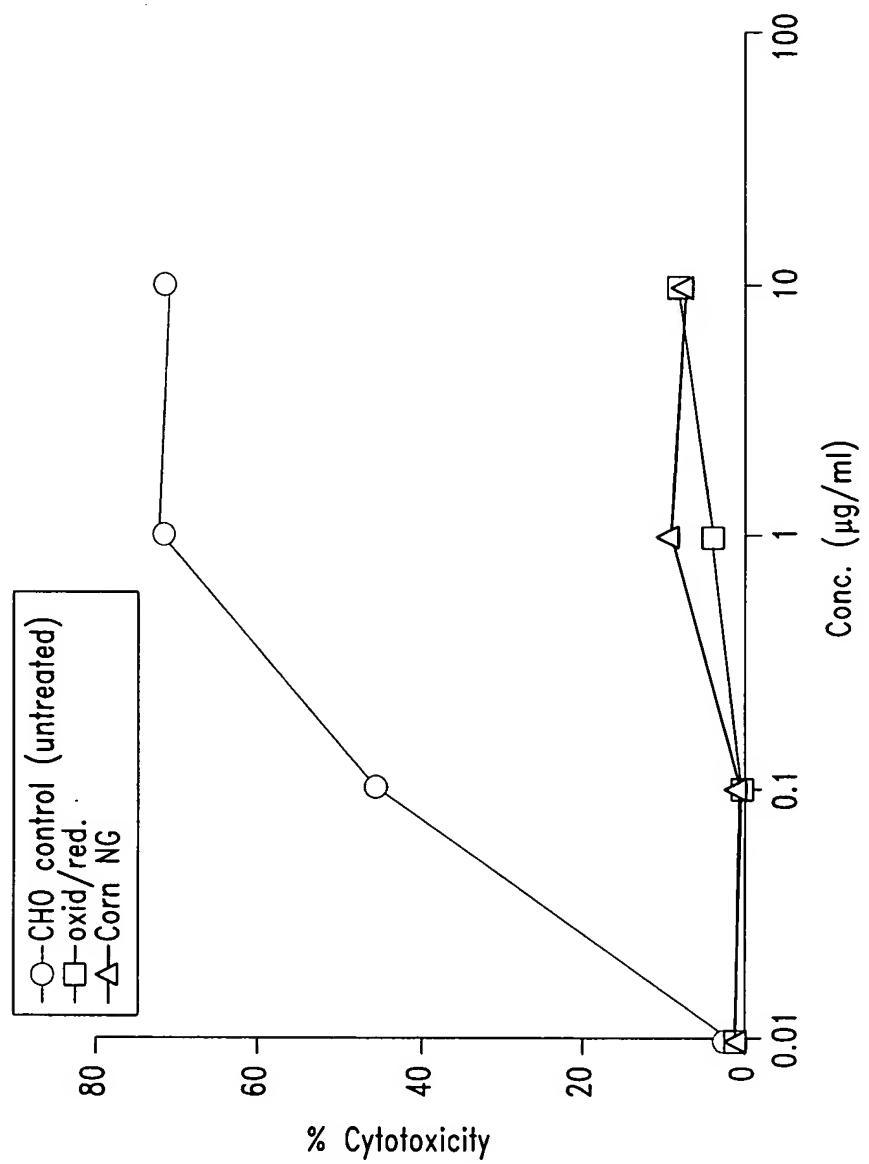


Fig. 15B

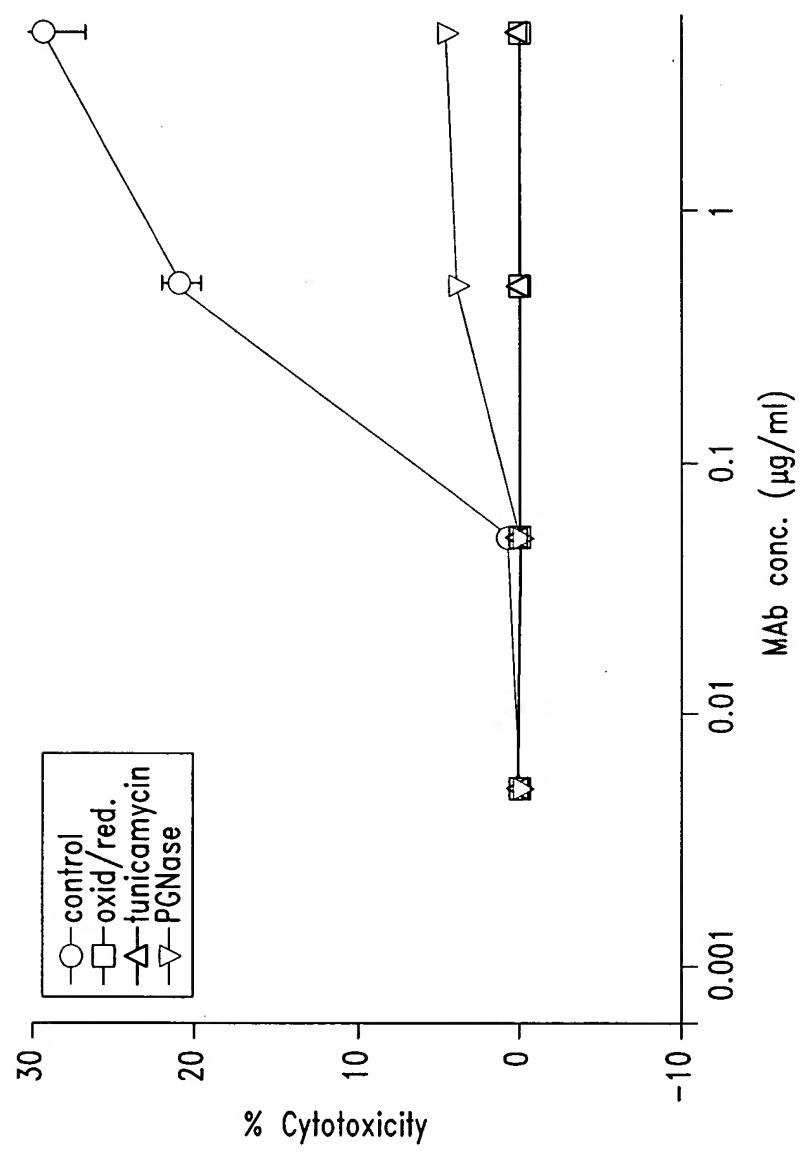


Fig. 15C

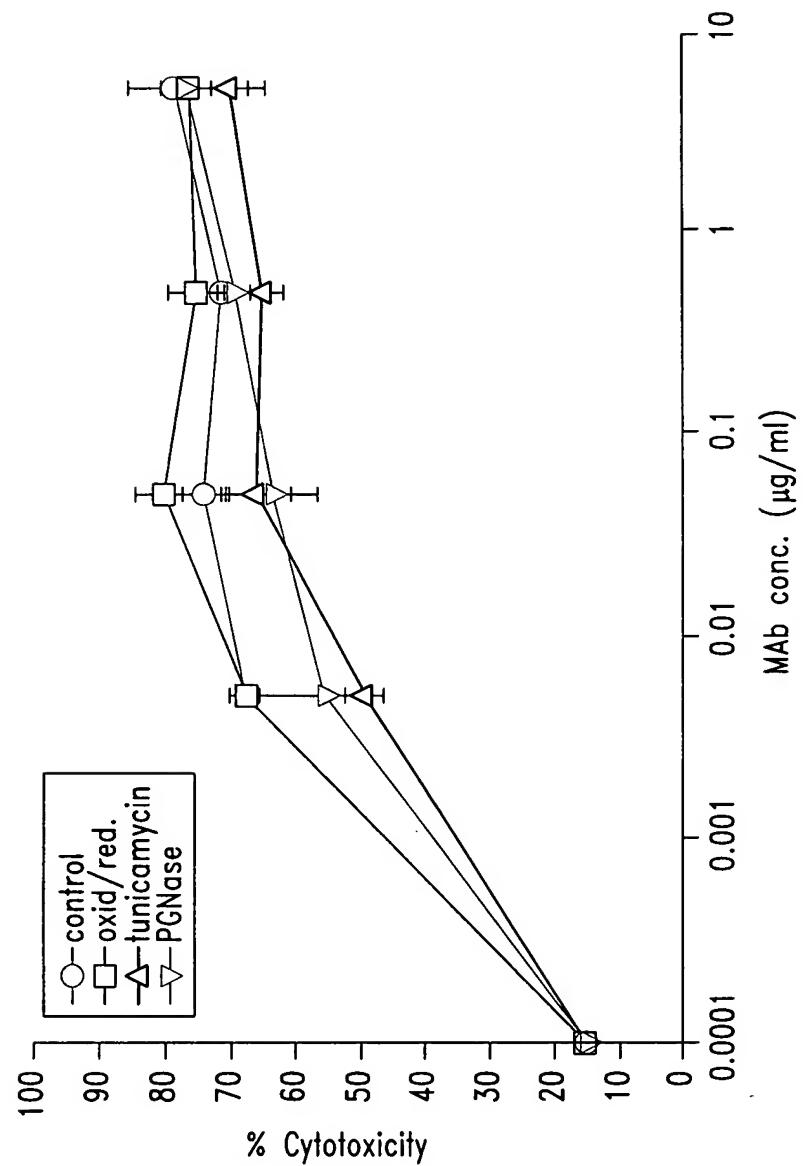


Fig. 15D

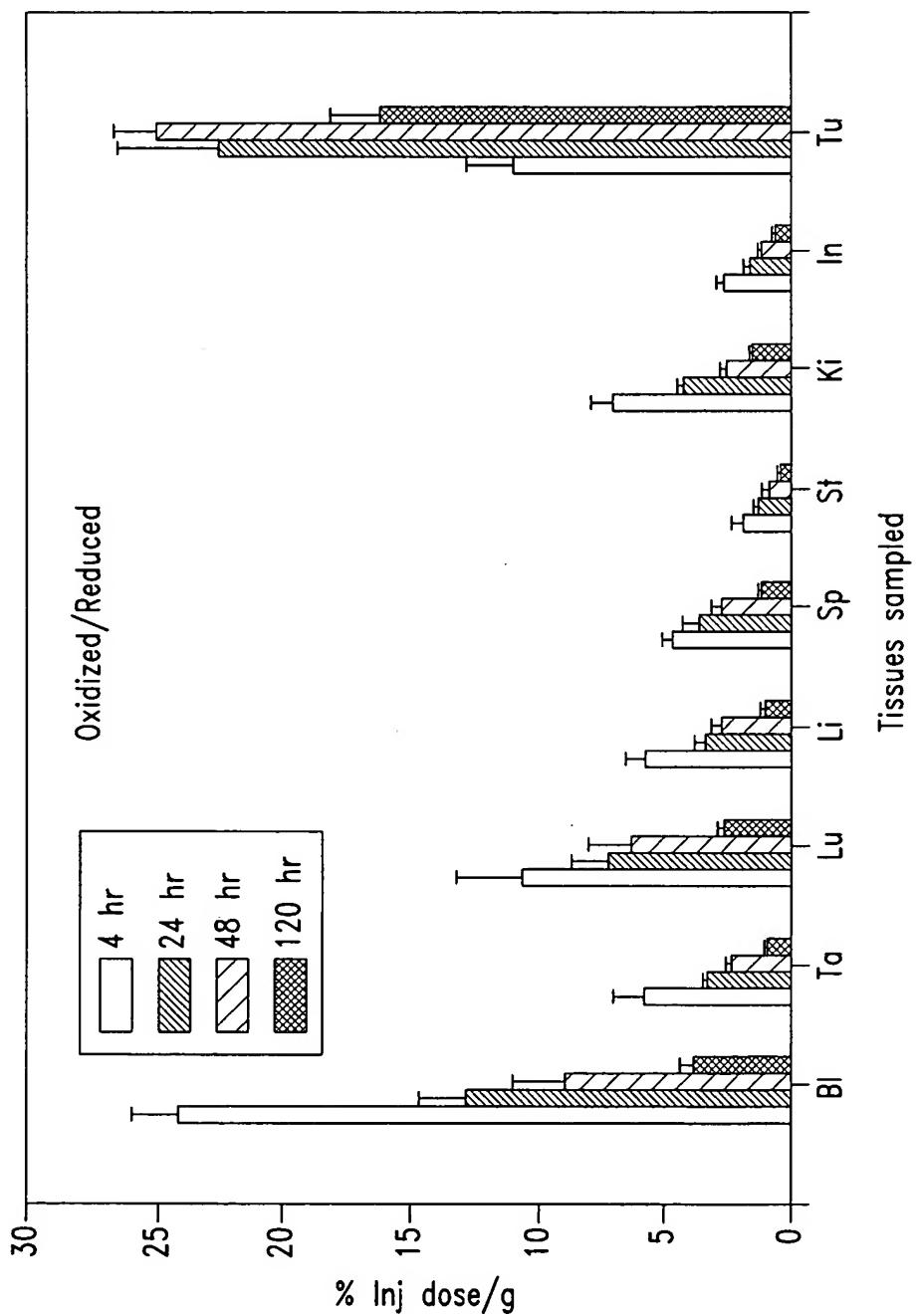


Fig. 16A

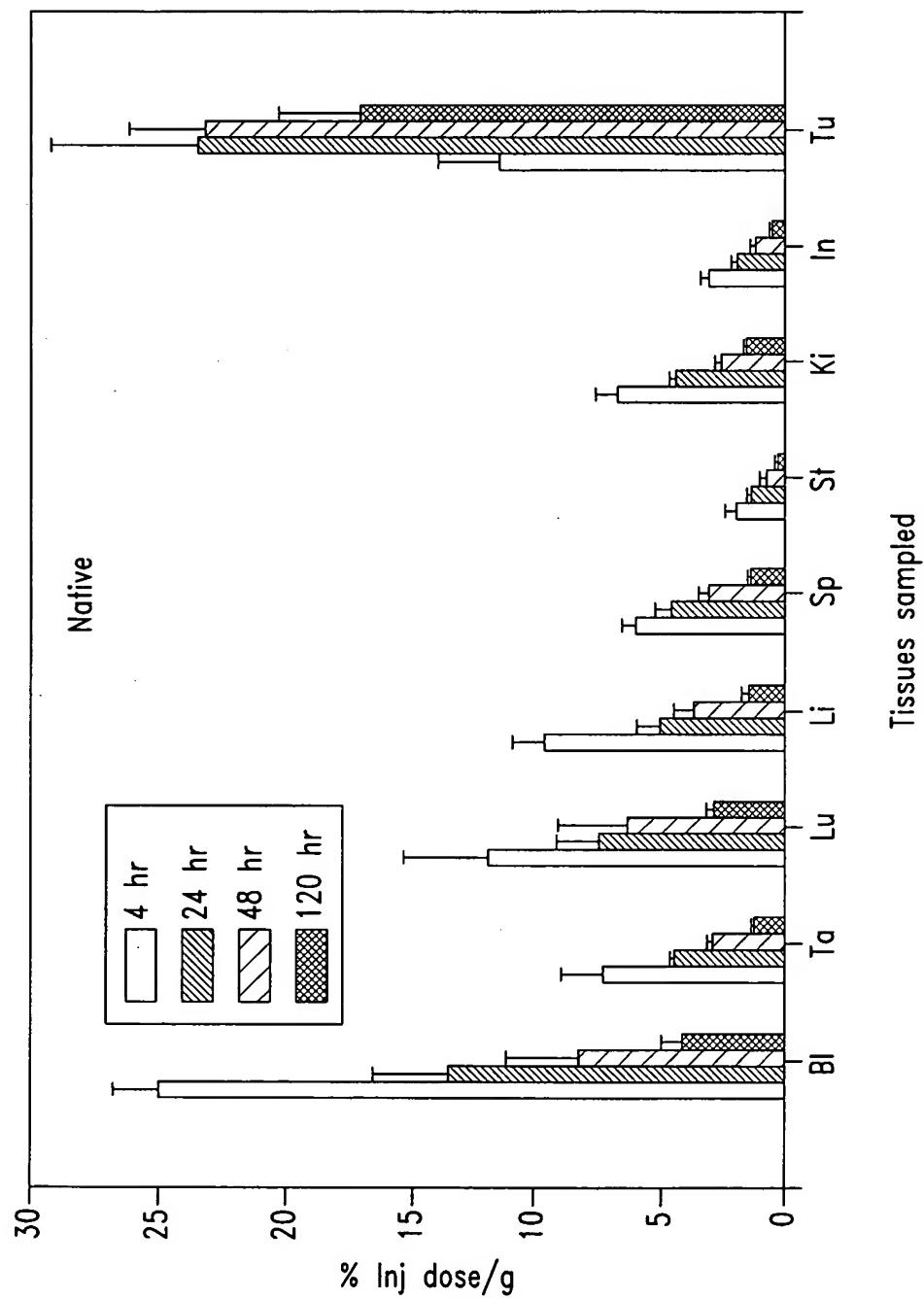


Fig. 16B

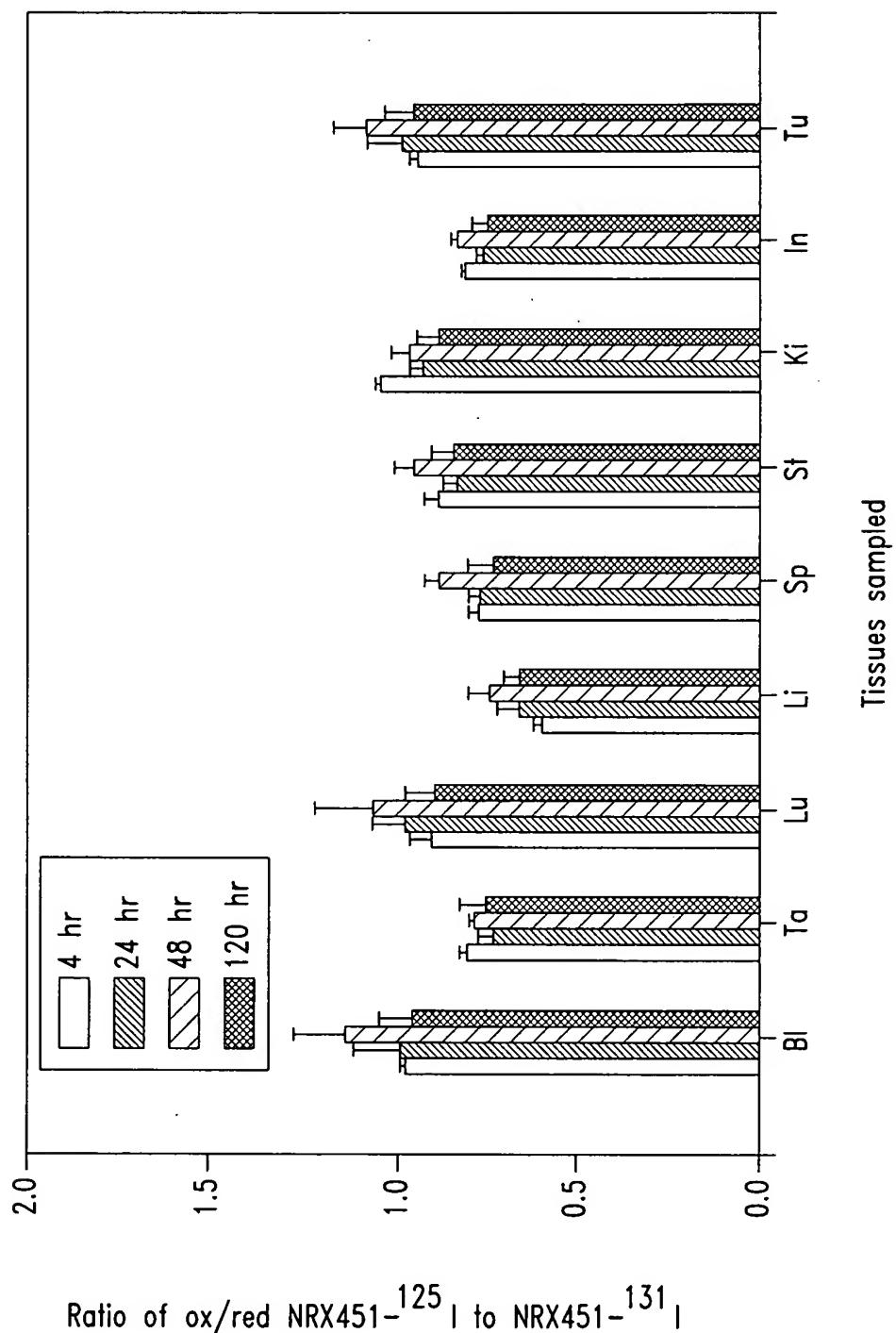


Fig. 16C

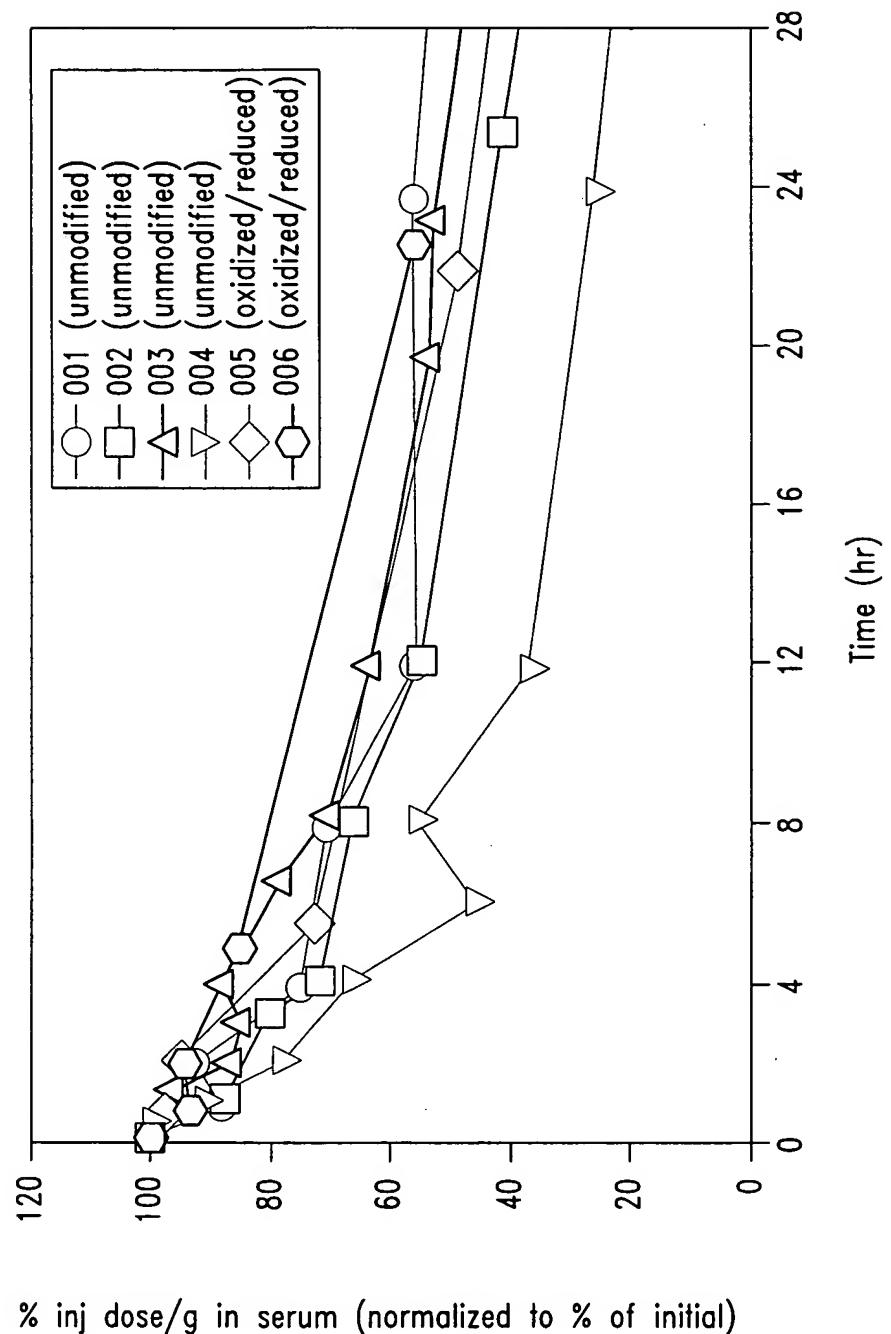


Fig. 17

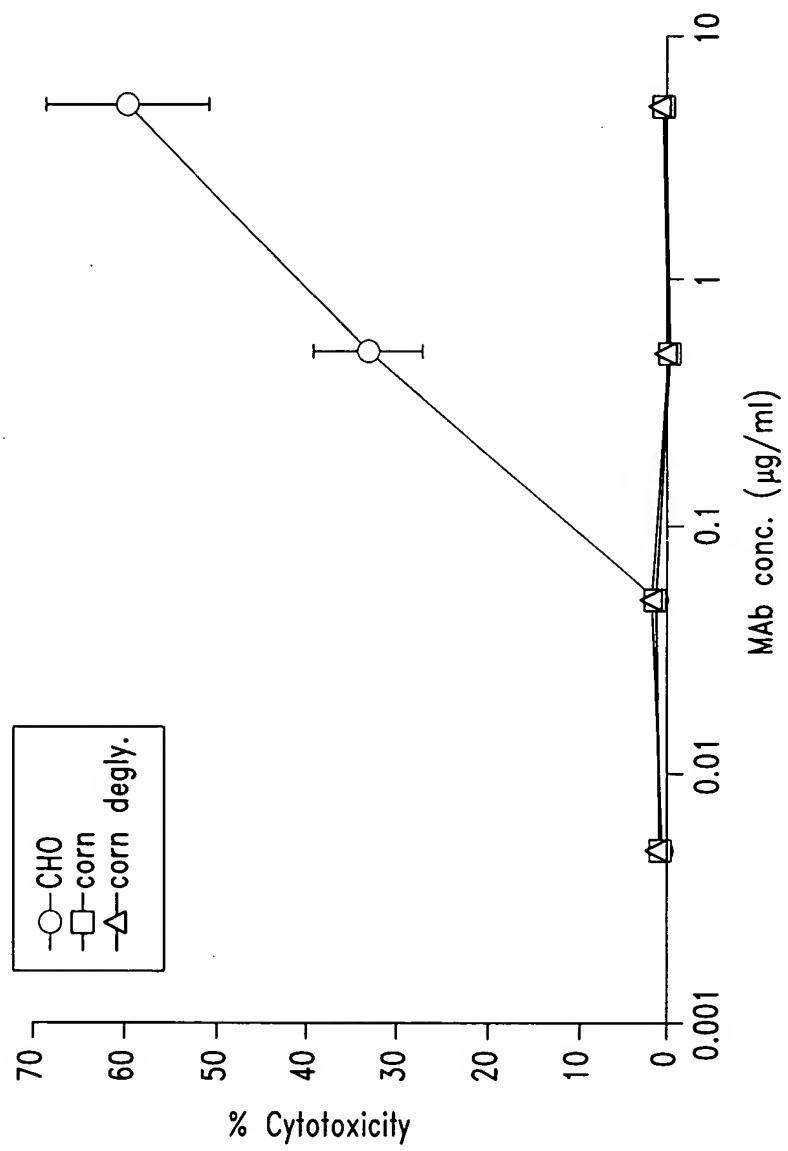


Fig. 18

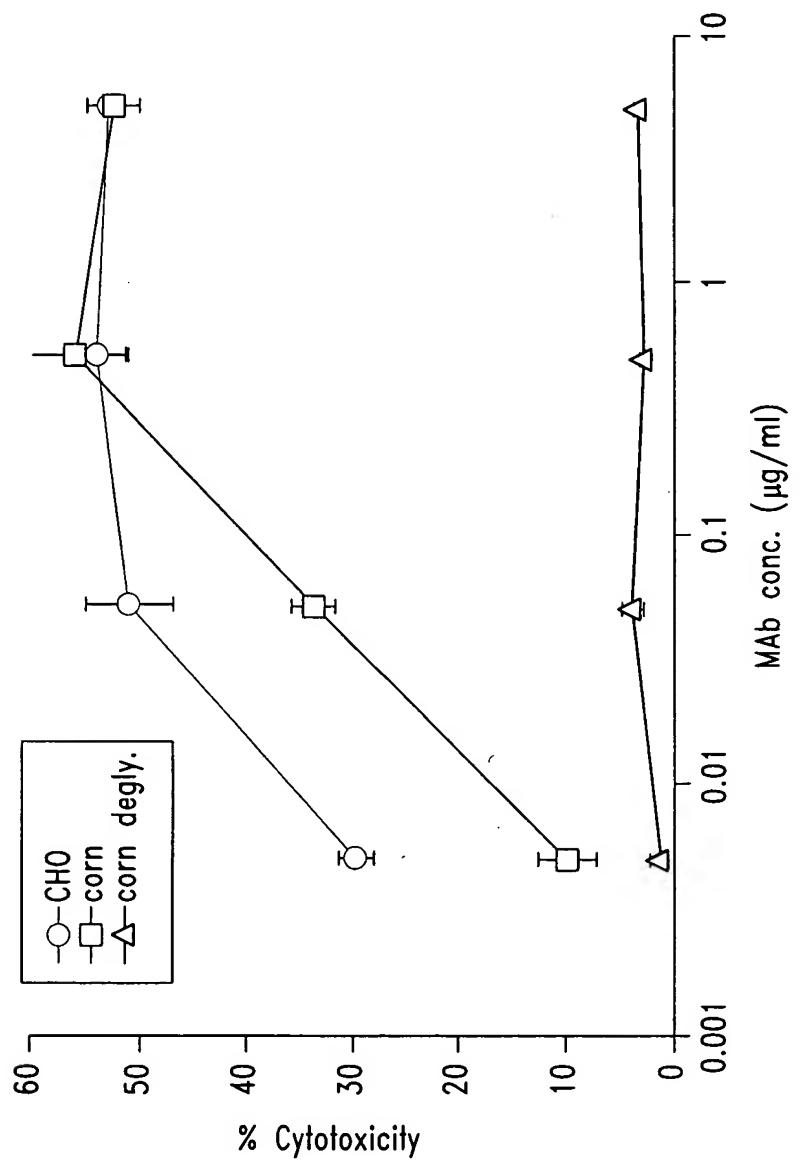


Fig. 19

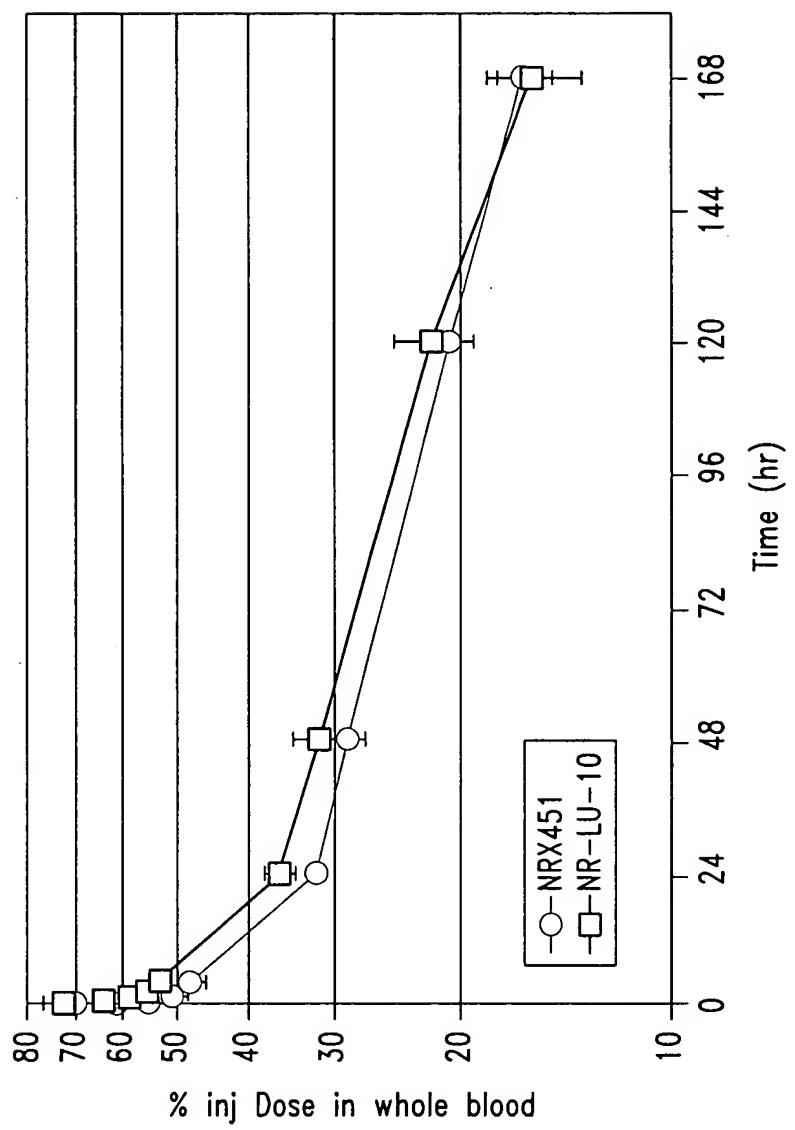


Fig. 20

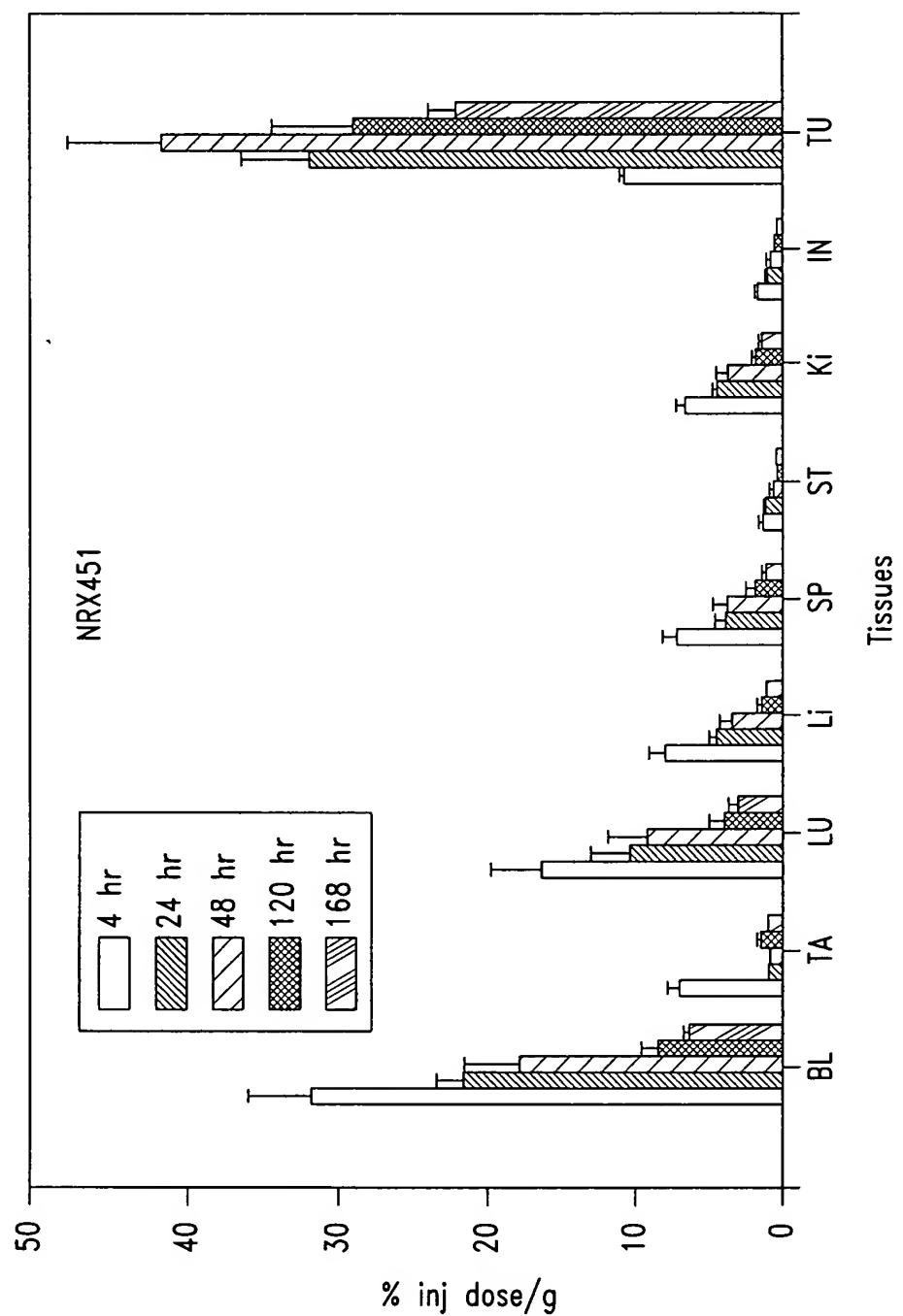


Fig. 21A

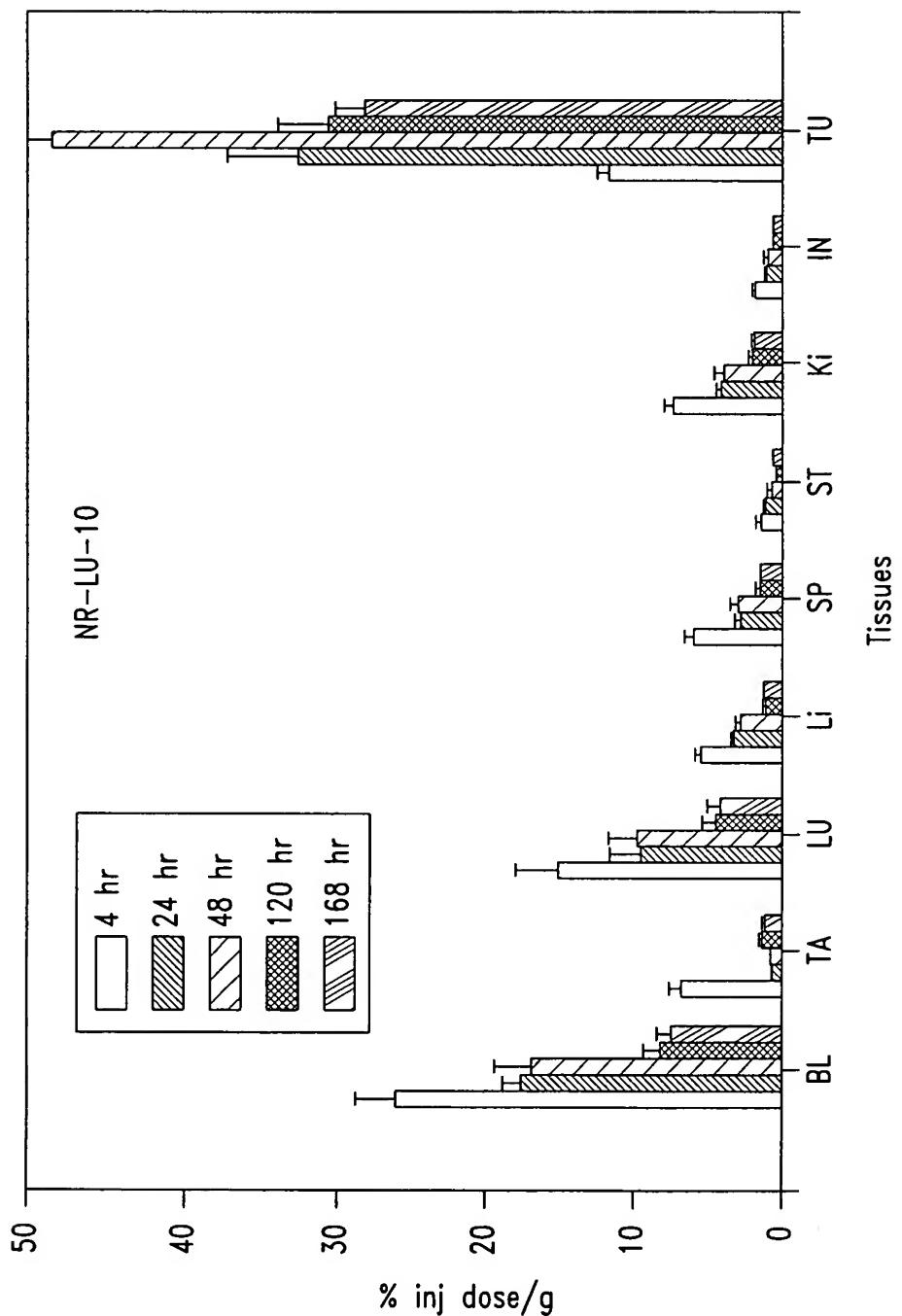


Fig. 21B

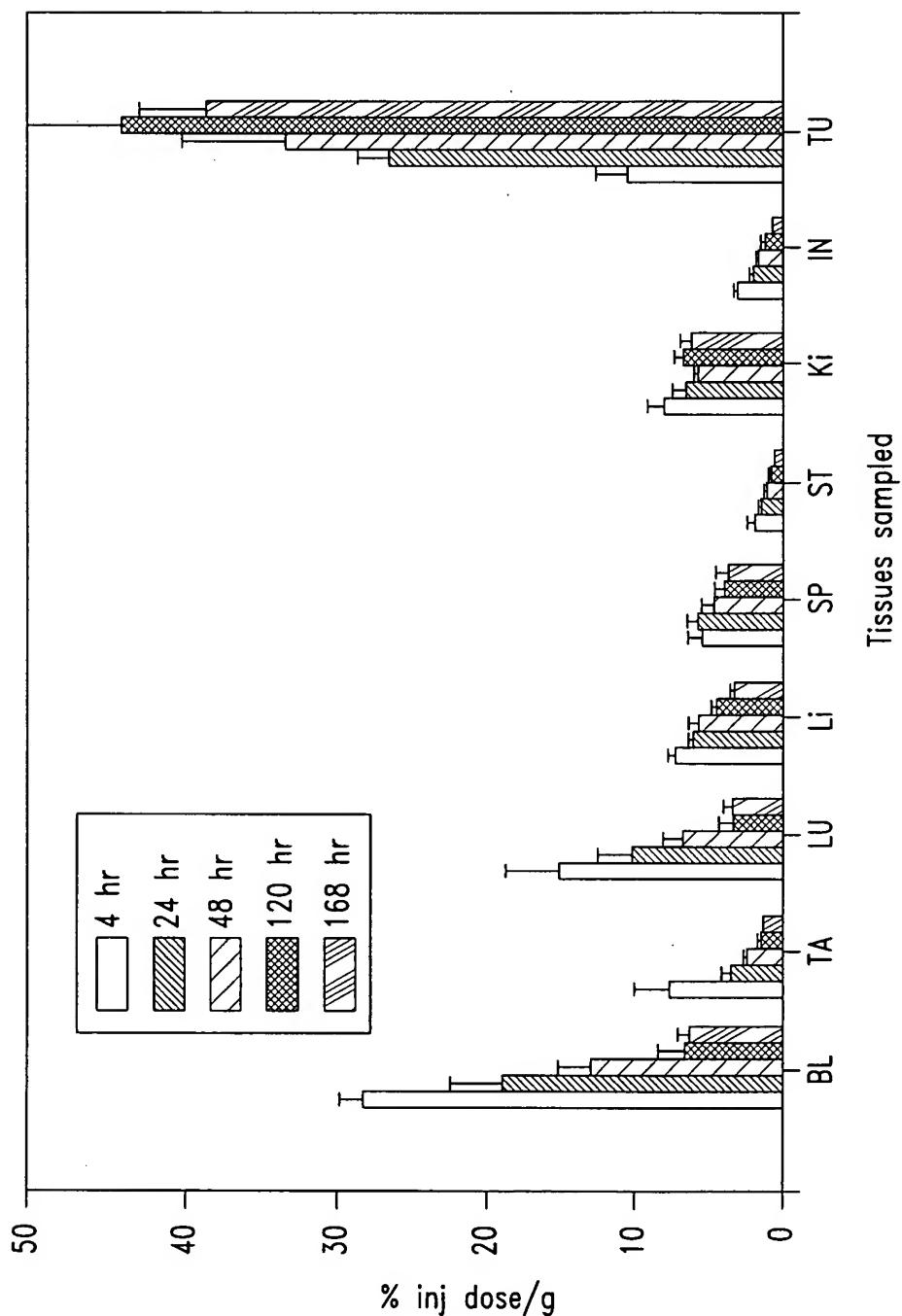


Fig. 22

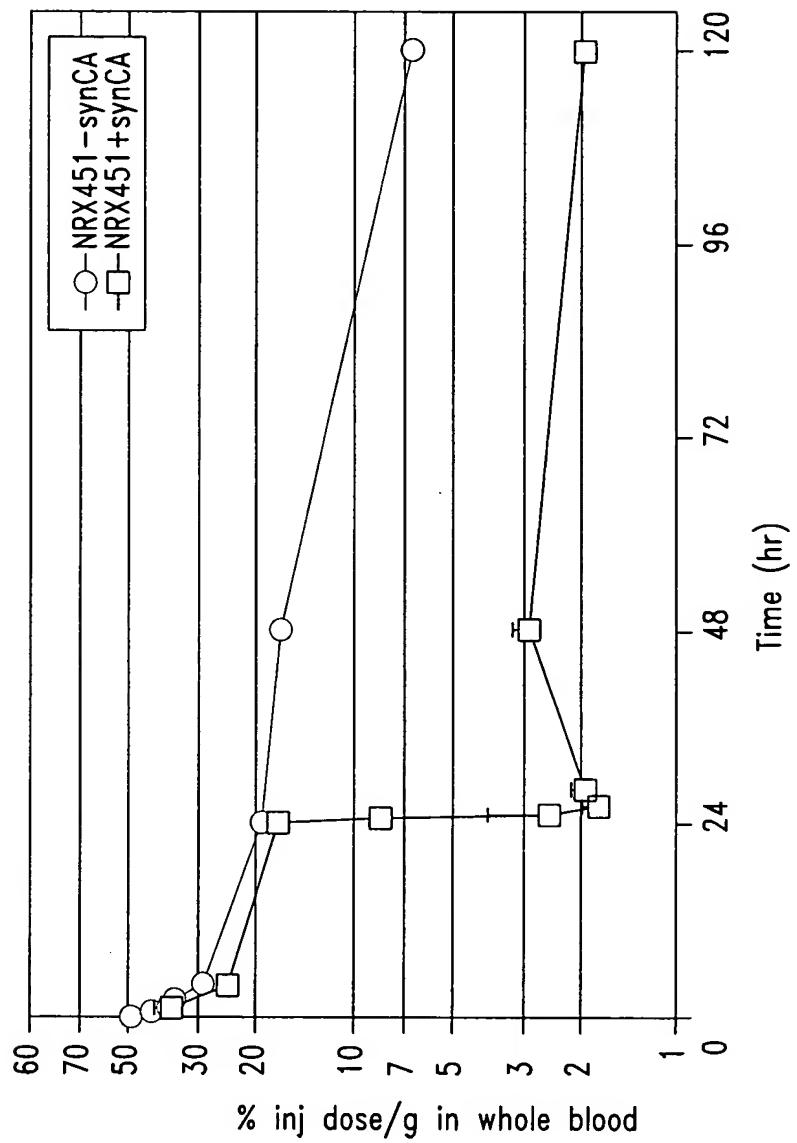


Fig. 23

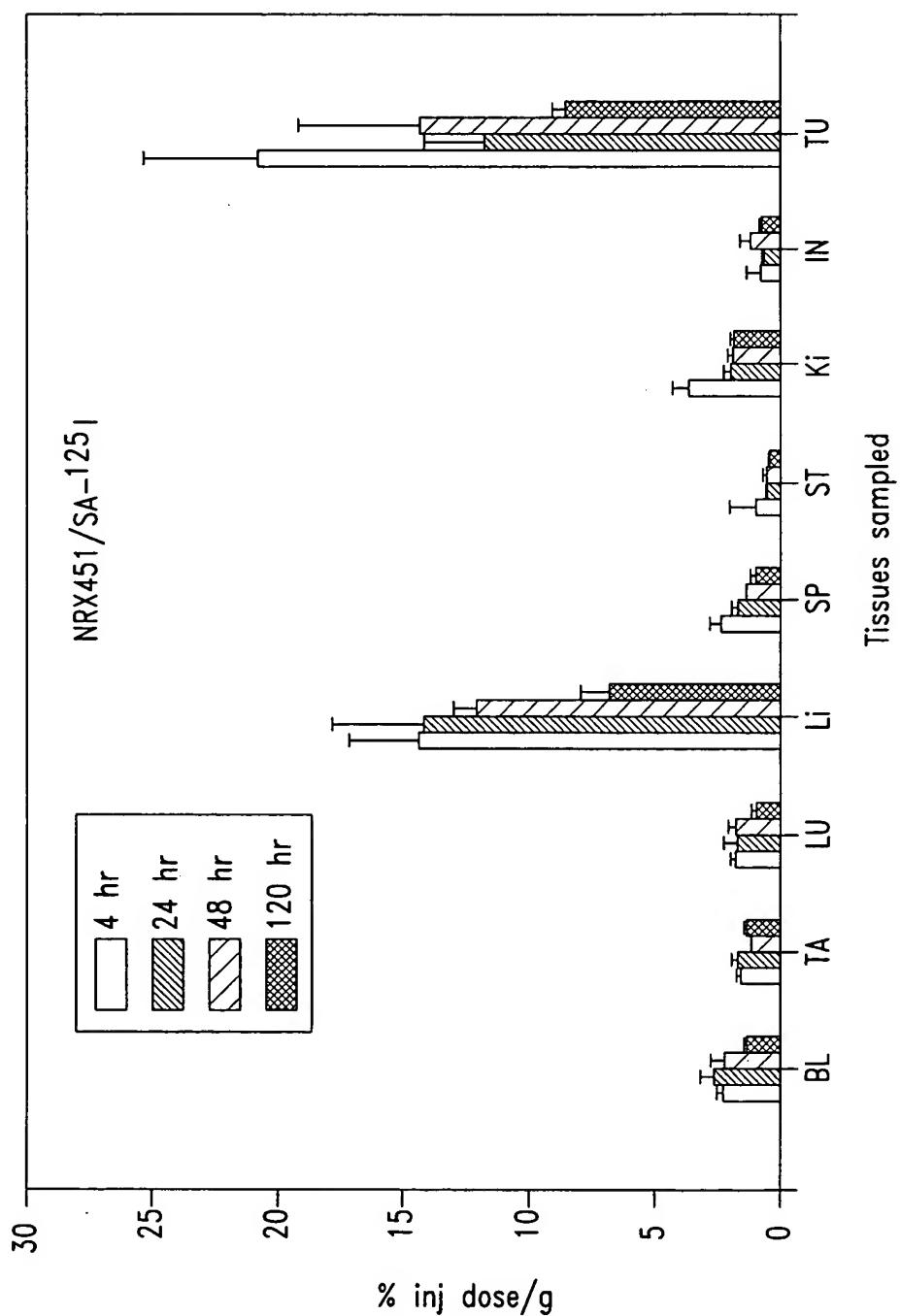


Fig. 24A

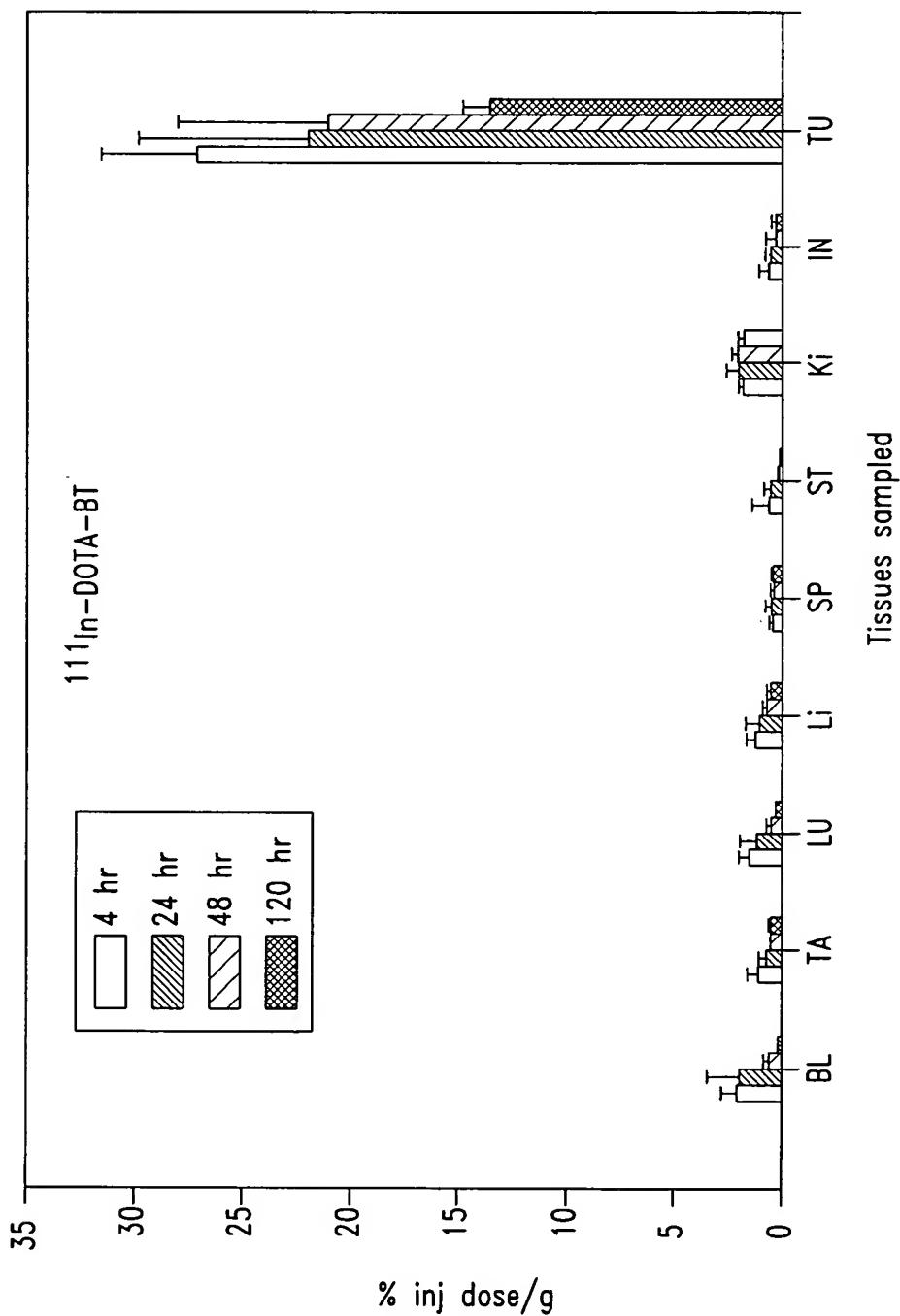


Fig. 24B